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Comparative Feline Genomics: A BAC/PAC Contig Map of the Major Histocompatibility Complex Class II Region

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The genome organization of the human major histocompatibility complex (MHC) will be best understood in a comparative evolutionary context. We describe here the construction of a physical map for the feline MHC. A large-insert domestic cat genomic DNA library was developed using a P1 artificial chromosome (PAC) with a genomic representation of 2.5× and an average insert size of 80 kb. A sequence-ready 660-kb bacterial artificial chromosome/PAC contig map of the domestic cat MHC class II region was constructed with a gene order similar to, but distinct from, that of human and mice: DPB/DPA, Ring3, DMB, TAP1, DOB, DRB2, DRA3, DRB1, DRA2, and DRA1. Fluorescence *in situ* hybridization analyses of selected class II PAC clones confirmed that the class II region lies in the pericentromeric region of cat chromosome B2. However, apparently unlike the human and mouse MHCs, the domestic cat DRA and DRB genes have undergone multiple duplications and the DQ region has been deleted.

INTRODUCTION

A hallmark of the mammalian major histocompatibility complex (MHC) is the extreme polymorphism of genes encoding the class I and II molecules, both within and between most species. The classical class I and II molecules are cell surface glycoproteins that bind, transport, and present small peptides derived from invasive parasites to T-cell receptors expressed on immune cells (Townsend and Bodmer, 1989; Zinkernagel, 1996). Class I molecules form heterodimers with β2-microglobulin and are expressed on all somatic cells. Class I molecules bind specific peptides in the endoplasmic reticulum and transport them to the cell surface. These peptides are presented to cytotoxic T-lymphocytes ($CD8^+$, $CD4^-$) that respond by granule exocytosis and/or fas-mediated target cell death (Smyth and Trapani, 1995, 1998). Class II molecules form heterodimers of α- and β-chains, encoded, for example, by DRA and DRB genes, respectively, that are expressed on antigen-presenting cells (e.g., macrophages or dentritic cells). Class II molecules bind peptides from endosomes and present these to T-helper cells ($CD4^+$, $CD8^-$) that generate an immune response in the form of cytokine release to stimulate B-cell antibody production and cytotoxic T-cell proliferation.

Human class I (HLA-A, -B, and -C) and class II (DRB, DQA, DQB, and DPB) genes have a degree of genetic variation unparalleled in the human genome (Hedrick and Thomson, 1983; Hedrick *et al.*, 1991; Hughes and Yeager, 1998; Gu and Nei, 1999; Parham, 1999; Yeager and Hughes, 1999); for example, HLA-B has 328 alleles, and HLA-DRB1 has 241 alleles (www.anthonynolan.com/HIG/). Comparative genetic analyses have established that extreme allelic variation in the MHC is a common feature among primate species (e.g., chimpanzee and rhesus macaque), rodents, agricultural animals, and companion animals, and certain allelic lineages of class I and II genes exhibit a transpecies mode of inheritance (Bergstrom *et al.*, 1999; Bontrop *et al.*, 1999; Chardon *et al.*, 1999; Gu and Nei, 1999; Lewin *et al.*, 1999; Satta *et al.*, 1999). The persistence of such extreme allelic variation is unusual compared to most gene polymorphisms and renders most individuals heterozygous at each locus (Kimura, 1983; Klein, 1986). Allelic diversity at mammalian MHC loci is maintained by balancing selection as demonstrated by the following: (i) nucleotide substitutions between alleles are predominantly nonsynonymous for amino acid residues in the peptide binding region (PBR) and synonymous for other regions of the mole-

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cule (Takahata and Satta, 1998a, b); (ii) the large number of alleles in a population; (iii) the large number of nucleotide substitutions among alleles; (iv) high heterozygosity; and (v) linkage disequilibrium between HLA loci (Huttley *et al.*, 1999; Klitz *et al.*, 1995). New alleles at class I and II loci are generated by both point mutation and gene conversion (Martinsohn *et al.*, 1999). Although a variety of selection scenarios have been proposed (e.g., disease resistance, reproductive incompatibilities, and mating preference), the most common source of diversity seems to involve historic outbreaks of pathogenic microbes as evidenced by genetic associations between MHC loci and the malarial parasite (Hill *et al.*, 1991), hepatitis B (Thio *et al.*, 1999), hepatitis C (Thursz *et al.*, 1999), human papilloma virus (Hildesheim *et al.*, 1998; Wank and Thomssen, 1991), and HIV/AIDS in humans (Carrington *et al.*, 1999); bovine leukemia virus in cattle and sheep (Nagaoka *et al.*, 1999; Xu *et al.*, 1993); and Marek's disease virus in chicken (Briles *et al.*, 1977, 1983).

The domestic cat has been subject to epidemics that have been traced to deadly viral pathogens, including feline infectious peritonitis virus, feline leukemia virus, an oncogenic retrovirus, and feline immunodeficiency virus, a lentivirus causing an immunodeficiency syndrome providing a natural animal model for AIDS (Carpenter *et al.*, 1996, 1998a, b; Carpenter and O'Brien, 1995; Evermann *et al.*, 1988; Hofmann-Lehmann *et al.*, 1996; Hoover and Mullins, 1991; Martinon and Levy, 1993; Roelke *et al.*, 1993; Roelke-Parker *et al.*, 1996; Willett *et al.*, 1997). These feline pathogens provide an opportunity to investigate the interaction between host immune response and infectious disease through studies of the MHC, T-cell receptor loci, immunoglobulin genes, natural killer receptor loci, and other genes that participate in the immune response.

Using alloantisera with overlapping serospecificities in domestic cats, Winkler *et al.* (1989) demonstrated that the specificities segregated as a single Mendelian trait, and they identified 24 MHC haplotypes. Screening of feline cDNA libraries using human class I and class II cDNAs allowed the cloning of feline cDNAs for class I and class II DRA and DRB, but failed to detect DQA, DQB, DPA, and DPB cDNAs (Yuhki *et al.*, 1989; Yuhki and O'Brien, 1997; Yuhki and O'Brien, unpublished results). Phylogenetic analyses of exon 2 (which encodes the PBR) from 61 DRB sequences across the three Felidae genera revealed at least five monophyletic lineages with abundant allelic variation. A twofold increase in the relative rate of synonymous versus nonsynonymous substitutions in the PBR versus non-PBR regions of class I genes and a similar tendency among class II DRB genes established the cat as the third mammalian species in which this pattern was observed (Yuhki and O'Brien, 1990, 1994, 1997; O'Brien and Yuhki, 1999). Polymorphic MHC genes are maintained by balancing selection for functional class II alleles (as are the class I genes) and a trans-species mode of evolution in Felidae with at least three cat DRB genes and two DRA genes per individual (Yuhki and O'Brien, 1997). The feline MHC has been mapped to chromosome B2 with a gene order of MOG–Class I–TNFA–CSNK2B–C4A–DRA–DOB–DPA–RPS18 (Yuhki and O'Brien, 1988; Murphy *et al.*, 1999, 2000).

The determination of gene order in the feline MHC was a direct result of technological advances propelled by the Human Genome Project that have recently advanced the construction of moderately dense gene maps of a variety of agricultural and companion mammals to make the era of comparative genomics a reality (O'Brien *et al.*, 1999). These technologies include the development of type I (coding genes) and type II (hypervariable microsatellite repeats or short tandem repeats (STRs)) markers, interspecies hybrid backcross panels for meiotic linkage mapping, radiation hybrid panels for rapid construction of dense physical maps, interspecies chromosome painting for identification of evolutionary conserved chromosomes, and bacterial artificial chromosome (BAC)/P1 artificial chromosome (PAC) libraries (Shizuya *et al.*, 1992; Ioannou *et al.*, 1994; Schmitt *et al.*, 1996; Solinas-Toldo *et al.*, 1995; Wienberg and Stanyon, 1995; Wienberg *et al.*, 1997; Womack and Kata, 1995; reviewed by O'Brien *et al.*, 1999). The availability of these maps will provide new animal model systems for study of the interaction of genes and environment with applications to human disease, perhaps including assessment of polygenic traits and pathologies, development of treatments for human disease, and the discovery of evolved adaptations to hereditary and infectious disease by genomic prospecting (O'Brien, 1995; O'Brien *et al.*, 1999). Advances in technological methods, robotics, and computer-assisted data analysis of nucleotide sequences have allowed the determination of complete, megabase-sized, nucleotide sequences including the complete nucleotide sequences of the (3.6 Mb) human MHC (The MHC Sequencing Consortium, 1999), (33.4 Mb) human chromosome 22 (Dunham *et al.*, 1999), the *Drosophila* genome (Adams *et al.*, 2000; Hoskins *et al.*, 2000), and the chicken (B locus, 90 kb) MHC (Kaufman *et al.*, 1999). Genomic DNA libraries constructed in BAC or PAC vectors were utilized in all these sequencing efforts because of their lower frequency of chimeric clones, higher transformation efficiency, and ease of purification from the host genome. These properties have made these vector systems the method of choice for positional cloning of disease genes, development of new genetic markers, bridging cytogenetic and genetic maps (i.e., meiotic or RH maps), and/or subsequent nucleotide sequencing of the clone (Boysen *et al.*, 1997; Cai *et al.*, 1998; Godard *et al.*, 1998; Green, 1997; Higgins *et al.*, 1998; Himmelbauer *et al.*, 1998; Kelley *et al.*, 1999; Korenberg *et al.*, 1999; Monaco and Larin, 1994). In this article, we describe the construction of a sequence-ready BAC/PAC contig map spanning 660 kb of the domestic cat MHC class II region and the construction of a feline PAC library.

MATERIALS AND METHODS

Construction of a feline P1 artificial chromosome library. pCYPAC3.0 vector (a generous gift from T. Ota, Boston University) was grown overnight in Luria broth (LB) medium containing 30 μg/ml kanamycin and 50 μg/ml ampicillin and purified using standard Triton-X lysis and two rounds of CsCl density gradient centrifugation (Sambrook *et al.*, 1989). Library construction followed the protocol of Amemiya *et al.* (1996). Briefly, 50 μg of vector was digested for 1 h at 37°C with *Bam*HI (New England Biolabs, Beverly, MA) at 0.5 units/μg in a 100-μl total volume following the manufacturer's recommendations. A 20-μl aliquot (10 μg) was diluted twofold by addition of 4 μl 10× calf intestinal phosphatase (CIP) buffer and 15 μl of H_2O, and 2.5 units calf intestinal phosphatase was added (40 μl total volume) and incubated for 1 h at 37°C. The reaction was terminated by the addition of EDTA to 10 mM, SDS to 0.5%, and protease K to 0.5 μg/μl and incubated at 56°C for 1 h. This was extracted twice with phenol:chloroform:isoamyl alcohol, precipitated by addition of NaOAc to 0.3 M and 2 vol EtOH, and washed with 80% EtOH. The precipitate was suspended in 100 μl TE and fractionated through a 0.8% agarose gel in 1× TBE to separate the vector from the pUC19 stuffer. The 16-kb vector DNA band was isolated by electrophoresis onto DE-81 paper (Beck *et al.*, 1990) and resuspended in TE at 20 ng/μl.

High-molecular-weight DNA from a male domestic cat fibroblast cell line (Fca 215) was prepared by embedding 5.3×10^7 cells per milliliter of molten insert agarose using plug molds (Bio-Rad Laboratories, Hercules, CA) at 75 μl/plug. The plugs were incubated in cell lysis solution for 16 h at 37°C, washed in 20% NDS (0.2% *N*-laurylsarcosine, 2 mM Tris–HCl, pH 9, 0.14 M EDTA) for 2 h at room temperature, and stored in fresh 20% NDS at 4°C. Plugs containing 10 μg domestic cat DNA were washed 2× for 30 min each at 4°C with 50 ml TE and then with 1× *Bam*HI buffer (without $MgCl_2$), individual plugs were equilibrated with *Bam*HI at 0.05, 0.1, 0.15, and 0.2 units/μg in a total volume of 250 μl of 1× *Bam*HI buffer (without $MgCl_2$) for 30 min on ice, and 2.5 μl of 1 M $MgCl_2$ was added, equilibrated for 30 min on ice, and then transferred to 37°C for 30 min. The digestions were terminated by addition of 1.25 ml of LDS (1% lithium dodecyl sulfate, 10 mM Tris–HCl, pH 8, 100 mM EDTA) for 30 min at room temperature. Pooled samples of *Bam*HI-digested domestic cat DNA were size-fractionated by equilibrating plugs in 50 ml of 1× TAE two times for 30 min at 4°C, removing the equilibration buffer, heating to 65°C for 10 min, and loading onto a 1% SeaPlaque GTG agarose gel, and electrophoresis was performed at 140 V with a linearly ramped switch time of 10 to 60 s overnight. Fractions containing DNA with an average size of 100, 200, and 300 kb were refractionated on a second 1% agarose gel as above. Gel slices containing size-fractionated domestic cat DNA were equilibrated two times in 50 ml of TE containing 50 mM NaCl for 1 h at 4°C. The plugs were then placed in 1.5-ml Eppendorf tubes and heated to 70°C for 15 min, transferred to 45°C for 15 min, and 1 unit β-agarose (Gibco BRL, Rockville, MD) was added per 100 μl and digested for 2 h. DNA concentration was estimated by electrophoresis through 0.8% agarose gels using λ DNA digested with *Hin*dIII as a standard. Ligation reactions contained 20 ng *Bam*HI-digested, CIP-treated pCYPAC3.0 vector, 20 ng cat DNA partially digested by *Bam*HI, 1× T4 DNA ligase buffer, and 200 units T4 DNA ligase (New England Biolabs). Ligation reactions were incubated overnight at 15°C and terminated by addition of EDTA to 20 mM and heating to 65°C for 2 min. DNA samples were drop-dialyzed on Millipore filters (type VS, 0.025 μm) for 2 h at room temperature against 100 ml of 0.5× TE, 3-μl aliquots were electroporated into 30-μl ElectroMax DH10B cells (Gibco BRL) at 1.2–1.4 kV using a GenePulser (Bio-Rad), and 1 ml of SOC medium was added and incubated at 37°C with shaking for 1 h. Then 200-μl aliquots were plated onto LB agar plates containing kanamycin (60 μg/ml), kanamycin (60 μg/ml) plus 5% sucrose, or kanamycin (60 μg/ml) plus ampicillin (50 μg/ml) to estimate yield and ratios of recombinants/nonrecombinants. The yields of recombinants obtained with 100-kb cat DNA inserts (3.3×10^5 colonies/μg) were of sufficient magnitude to generate a cat genomic PAC library. Therefore, the ligation reactions were scaled up to a 400-μl total volume using the 100-kb fraction of cat DNA, and the reaction products were electroporated into *Escherichia coli* DH10B cells. The library was plated onto Nunc bioassay (22 × 22 cm) dishes containing LB agar kanamycin (60 μg/ml) plus sucrose 5% at 10 colonies/cm^2 and incubated for 12 h at 37°C until the colony size was 0.5 mm. The plates were harvested by scraping into SOC medium, pooled, and stored in SOC medium containing 10% glycerin at −70°C.

The average insert size of the PAC library was determined by analysis of 35 randomly picked PAC clones. PAC DNAs were prepared as described by Amemiya *et al.* (1996) and digested with *Not*I (NEB) using conditions specified by the manufacturer. The products were analyzed by pulsed-field gel electrophoresis as described above. The genomic representation was determined according to the formula of Clarke and Carbon (1976).

For colony lifts, the amplified library was plated onto LB agar bioassay dishes as above except that the library was plated at 50 colonies/cm^2 and grown overnight at 37°C; colonies were lifted in duplicate onto nylon membranes (Stratagene, La Jolla, CA), and the membranes were placed on LB agar dishes as described above containing 50 μg/ml IPTG and grown until colonies were 0.5 mm in diameter. The membranes were treated with denaturing solution (0.5 N NaOH/0.5 M NaCl) for 10 min, treated with neutralizing solution (0.5 M Tris–HCl, pH 7.5, 0.5 M NaCl) for 10 min, allowed to air-dry, baked at 70°C under vacuum, and UV cross-linked using a Stratalinker (Stratagene). To remove cellular debris, the membranes were incubated in hybridization solution overnight at 65°C and washed in 2× SSC/0.1% SDS (30 min) and 0.2× SSC/0.1% SDS (30 min) at 65°C.

Feline BAC library. To increase the feline genomic representation, a second feline genomic DNA library (designated RPCI 86) prepared from peripheral blood mononuclear cells of a male domestic cat (Fca 273) in pTARBAC and arrayed into 384-well plates with funding from the Ralston/Purina Co. (Wang *et al.*, manuscript in preparation) was screened. RPCI 86 consists of 234,349 clones with an average insert size of 137 kb and a 10.6-fold redundancy. BAC clones received as stab cultures were scraped using a sterilized pipet tip and inserted into wells of a 96-deep-well plate containing 250 μl LB/10% glycerol/25 μg/ml chloramphenicol. The deep-well plates were incubated for 16 h at 37°C with mild shaking, and 25 μl was streaked onto individual 100-mm petri dishes containing Luria agar/25 μg/ml chloramphenicol (Teknova) and grown for 16 h at 37°C. The 96-well plate was stored at −70°C as a freezer stock.

DNA and RNA probe preparation. DRA and DRB were isolated from plasmids pRA1 (Fca 51576, DRA*0101) and pRB3 (Fca 51574, DRB*0214) by digestion with *Bam*HI and *Hin*dIII, fractionated by electrophoresis through 1% agarose, and then isolated by electrophoresis onto DE-81 paper as described above. PCR primers were designed using the Primer 3 program (Rozen and Skaletsky, 2000) for domestic cat DPA, forward 5′-CCACGTGTCAACGTATGTCC–3′ reverse 5′-GGACCGTTGGATCAGAGTGT–3′ (derived from domestic cat DPA pseudogene (Verhoeven *et al.*, 1988); DOB3′ forward, 5′-ATGGGAACCAGAACTACCCC-3′, and reverse, 5′-GACACCAC-CGAGACTTCCAT, 413-bp amplicon (derived from 6B1catDOB); FTAP1ae4, forward, 5′-GCTGAGCCTGTTTTTGTGGT-3′, and reverse 5′-TACCTGGTACCATTTCCCCA-3′, 148-bp amplicon (derived from partial nucleotide sequencing of 6B1catDOB); DMB, forward, 5′-TCTCATGGAGGAAGAATGGG-3′, and reverse, 5′-CACATGCAC-CACAACATCAA (derived from partial nucleotide sequencing of PAC F23); and Ring 3 (FDP24), forward, 5′-GAACACGAAAAGTGGTT-TAGGG-3′, and reverse, 5′-CATCCGTTTGGAAATGGC-3′, 191-bp amplicon (derived from partial nucleotide sequencing of PAC F20). PCR was carried out using *Taq* Gold as described by the manufacturer (PE Applied Biosystems, Foster City, CA) and touchdown PCR (10 min at 96°C: 6 cycles of 30 s at 94°C, 30 s at 60°C, 30 s at 72°C; 1 cycle of 30 s at 94°C, 30 s at 59°C, 30 s at 72°C; 1 cycle of 30 s at 94°C, 30 s at 58°C, 30 s at 72°C; 1 cycle of 30 s at 94°C, 30 s at 57°C, 30 s at 72°C; 1 cycle of 30 s at 94°C, 30 s at 56°C, 30 s at 72°C; 25 cycles of 30 s at 94°C, 30 s at 55°C, 30 s at 72°C; with a final extension of 10 min at 72°C). PCR products were isolated as above

except that the gel concentration was 2% agarose. Isolated DNA products were radiolabeled by using a Random Priming Kit (Boehringer Mannheim, Indianapolis, IN) and [^{32}P]dCTP according to the manufacturer's instructions, and unincorporated nucleotides were removed by spun G-50 columns (Boehringer Mannheim). Riboprobes were synthesized using T7 and SP6 riboprobes as described by the manufacturer (New England Biolabs) using 1 μg of *Eco*RI-digested BAC/PAC DNA in a total volume of 10 μl, and unincorporated nucleotides were removed as above.

Library screening and Southern blot hybridizations. Nylon or Duralon UV membranes (Stratagene) were incubated in prehybridization solution (Gibco BRL) at 65°C for 2 h. ^{32}P-labeled DNA/RNA probes prepared as described above were denatured for 10 min in a boiling water bath or in certain cases prehybridized with domestic cat Cot-1 DNA (250 μg/ml) prepared as described by Nisson *et al.* (1991) or total cat DNA (2.5 mg/ml) and prehybridized as described by Sealy *et al.* (1985), mixed with hybridization solution (Gibco BRL) at 0.5–1 $\times$ 10^6 cpm/ml, hybridized overnight at 65°C, washed three times with 2$\times$ SSC at room temperature, washed three times with 2$\times$ SSC/0.1% SDS (30 min) at 60°C, and rinsed once with 2$\times$ SSC at room temperature. The filters were wrapped in Saran Wrap, exposed to X-ray film, and processed as described previously (Beck *et al.*, 1990).

High-throughput BAC/PAC DNA Minipreparations. High-throughput BAC/PAC DNA minipreparations were performed in a 2-ml 96-deep-well plate format using 1.25 ml of Luria broth, supplemented with the appropriate antibiotic (PACs with 30 μg/ml kanamycin or BACs with 25 μg/ml chloramphenicol), and two 3.5-mm glass beads/well, essentially as described by Ng *et al.* (1996). Three colonies/clone were picked with a sterile toothpick, inoculated into wells of the 96-well plate, and grown for 20 h at 37°C in a shaking incubator. Briefly, after the cells were pelleted by centrifugation in a Sorvall RT6000 at 3000 rpm for 10 min at 4°C and drained onto a paper towel, the bacterial cells were resuspended in 100 μl of P1 buffer (Qiagen, Valencia, CA) containing RNase (50 μg/ml) instead of GTE and lysed with 200 μl of P2 (Qiagen), and the bacterial nucleoid was removed by addition of 150 μl P3 (Qiagen) and centrifugation as above. The supernatant ($\sim$400 μl) was transferred to a 1-ml 96-deep-well plate precipitated with 280 μl isopropanol and centrifuged as above for 20 min. The precipitate was washed with 80% EtOH, dried under vacuum, and resuspended overnight in 50 μl TE (without RNase) overnight at 4°C prior to Sephacryl S-500 chromatography (Amersham Pharmacia Biotech, Piscataway, NJ) using a 96-well Silent Screen Plate (Nalge Nunc International, Naperville, IL). The yields were 100–200 ng for BAC DNA and 500–1000 ng for PAC DNA (induced for 3 h with 1 mM IPTG).

Restriction fragment fingerprint analysis. Restriction fragment fingerprint analysis was carried out essentially as described by Marra *et al.* (1997, 1999). Briefly, 25 μl of BAC or 10 μl PAC DNA was digested in triplicate with 20 units *Hin*dIII in a total volume of 50 μl as directed by the manufacturer (New England Biolabs) for 4 h at 37°C in a 0.2-ml 96-well plate. The digested BAC/PAC DNAs were precipitated by addition of 5 μl of 3 M NaOAc, pH 5.5, and 120 μl EtOH, chilled on ice for 10 min, centrifuged as above, and washed with 100 μl 80% ETOH, and then the plate was centrifuged inverted on a paper towel, until the speed reached 1500 rpm. The plate was then dried *in vacuo* for 10 min, the pellets were suspended in 6 μl of 1$\times$ TAE loading dye (Sambrook *et al.*, 1989), and heated to 60°C for 5 min. The entire sample was fingerprinted by electrophoresis through 0.5-mm-thick 1% agarose gels (50-well comb) in special ordered 20 $\times$ 25 cm electrophoresis units (Owl Separation Systems, Inc., Portsmouth, NH) with recirculating 1$\times$ TAE maintained at 14°C using a CHEF DRII Chiller System (Bio-Rad Laboratories) at 70 V for 16 h. Agarose gels were stained in 500 ml of a 1:10,000 dilution of SYBR Green (Molecular Probes) in 1$\times$ TAE for 1 h, rinsed with H_2O, and imaged using a Hitachi Fluorimager set at 300 dpi, 16 bits, reading sensitivity 100%, and a 505-nm filter. Insert sizes of BAC/PAC DNAs were estimated from the *Hin*dIII fingerprints using FMBIO Analysis software package. Gel images were transferred to a DEC alpha (FCRDC Supercomputing Facility) using the raw data setting in Fetch and imported into IMAGE V3.9a; the normalized band positions from each clone were extracted and sent to FingerPrinting Contigs (FPC V3.2.1) (Soderlund *et al.*, 1997). Automated contig construction was performed essentially as described by Waterston and colleages (see Marra *et al.*, 1999) using FPC Eq. [1] (Soderlund *et al.*, 1997; Sulston *et al.*, 1988) a probability of coincidence score of 10^{-9}, a tolerance of 5, diffbury at 0.1, and minbands at 3 (unless otherwise indicated). For manual contig construction, the cutoff score was reduced to 10^{-8} unless indicated otherwise. After imaging, the fingerprints were transferred to Duralon UV (Stratagene) using standard protocols (Sambrook *et al.*, 1989) for use as hybridization tools. Contig verification included (1) reanalysis using *Bam*HI fingerprints, (2) side-by-side comparison of *Hin*dIII fingerprints predicted to be overlapping, (3) Southern blot or colony hybridization, and/or (4) PCR.

Fluorescence in situ hybridization (FISH). PAC DNA was labeled with biotin-11 (Sigma) by nick-translation (Rigby *et al.*, 1977). The final probe was 200–400 bp in size as determined by electrophoresis through 1.2% agarose. Metaphase spreads of a domestic cat cell line (Fca 215) were prepared by standard cytogenetic techniques and fluorescence *in situ* hybridization as described previously (Modi *et al.*, 1987; Lichter *et al.*, 1990). Briefly, metaphase spreads were denatured in 70% formamide/2$\times$ SSC at 80°C for 90 s and dehydrated in cold 70, 90, and 100% EtOH. Biotinylated DNA probes (400 ng), salmon sperm DNA (10 μg), and domestic cat Cot-1 DNA were suspended in 50% formamide/10% dextran sulfate/2$\times$ SSC (10 μl), denatured for 10 min at 75°C, layered onto denatured metaphase chromosomes, and hybridized for 48 h at 37°C. The metaphase spreads were washed and blocked, and the hybridized biotinylated probe was detected using fluorescein isothiocyanate (FITC)-conjugated avidin DCS (5 mg/ml, Vector Laboratories). Fluorescence signals were captured as grayscale images using a Zeiss Axiokop epifluorescence microscope equipped with a cooled charge coupled device camera (Photometrics CE 250) and merged with images of diamidinophenylindole (DAPI)-stained chromosomes using the Oncor Image system and software.

RESULTS

Feline PAC Library Analysis

A feline genomic DNA library was constructed in pCYPAC3.0 using *Bam*HI-digested cat genomic DNA from fibroblast cell line Fca 215, which was previously used to generate a domestic cat radiation hybrid panel (Murphy *et al.*, 1999). SucR colonies were obtained in an insert-dependent manner with 100-kb inserts with nonrecombinant/recombinant ratios of 0.8–1.6%. The total yield of recombinants was 91,900 as determined by pooling all the recombinants on kan + suc plates. To determine the insert size of the cat genomic library, 35 clones were digested with *Not*I and analyzed by PFGE. The average insert size was approximately 80 kb. The genomic representation is estimated at 2.5$\times$ coverage based on the insert size, the number of recombinants, and 3 $\times$ 10^9 bp/haploid genome, and the probability of finding any given genomic segment is 0.91 (Clarke and Carbon, 1976). To estimate the actual library representation, we screened the DNA prepared from a 1$\times$ complement of the pooled library with 52 comparative anchor tagged sequences (CATS) using polymerase chain reaction (Lyons *et al.*, 1997) and compared the PCR products obtained from PAC library with cat genomic DNA by agarose gel electrophoresis. The results indi-

cated that 43 of the 52 CATS were represented in the cat genomic PAC library, and therefore, the actual probability of finding any given gene is 83% (data not shown).

Isolation of Feline MHC Class II PAC Clones

To isolate genomic clones containing the feline MHC class II region, the PAC library was screened sequentially with domestic cat DRA and DRB cDNAs (Yuhki and O'Brien, 1997), PCR products representing DOB (exon 2 and 3 sequences amplified from domestic cat DNA; Yuhki and O'Brien, unpublished results), and the DPA pseudogene (Verhoeven *et al.*, 1988), Ring 3 (derived from partial nucleotide sequences from PAC F20), and T7 and SP6 riboprobes for PACs F2 and F3. A total of 11 positive colonies were confirmed as positive, assuming an average insert size of 80 kb; the target size of this screen (11 PACs $\times$ 80 kb = 880 kb/2.5 genome-equivalents) was 352 kb of feline MHC class II.

Isolation of Feline Class II Region BAC Clones

During the course of this work, a second feline genomic DNA library, designated RPCI 86, was constructed in pTARBAC (Wang *et al.*, manuscript in preparation). A 4.8$\times$ component of this library (Segment I) was screened with probes for DRA, DRB, DOB5$^-$, TAP1, and DMB (prepared as described under Materials and Methods). A total of 22 class II-positive BAC clones were confirmed as positive for one or more probes, assuming an average insert size of 138 kb per clone; the target size of this screen (22 BACs $\times$ 138 kb/4.8 genome-equivalents) was 632.5 kb of feline MHC class II. However, only one BAC clone (51h7) contained TAP1-related sequences, and therefore, a subset of clones from Segment II (5.8$\times$) was screened with TAP1, which identified BACs 463h11 and 439p14.

Fingerprint Analysis of Feline MHC Class II BAC/PAC Clones

Having identified 24 feline BAC clones and 11 feline PAC clones that were positive by hybridization for nucleotide sequences that map to the mammalian MHC class II region, we sought first to order the feline clones into contigs using established high-throughput fingerprinting techniques. We elected to use the agarose gel system described by Waterston and colleagues (see Marra *et al.*, 1997, 1999) because not only has it been proven to order clones into contigs with a high degree of accuracy, but also because the fingerprints could be transferred to membranes and used in hybridization experiments that might aid in ordering genes that have evolved by the process of gene duplication. Therefore, BAC/PAC DNAs were prepared in triplicate, digested with *Hin*dIII, and fingerprinted using 1% agarose gels as described under Materials and Methods. Fluorescently stained gel images were collected and analyzed for restriction fragment identification, normalized restriction fragment mobility, and size estimation in basepairs. Computer-assisted analysis using FPC software (Soderlund *et al.*, 1997) allowed us to determine the probability of coincidence scores for each clone relative to all other clones in the analysis and thus to identify overlapping clones.

The calculated pairwise probability of coincidence scores for each BAC clone against all BAC clones indicated that the 24 BAC clones could be arranged into three BAC contigs (BACs 181p11-244j14, BACs 69c13-51h7, and BACs 463h11-102h1) using automated assembly with a cutoff score of $<1 \times 10^{-9}$, and second, manual assembly using a cutoff score of $<1 \times 10^{-8}$ allowed the three contigs of BAC clones to be assembled into one contig as illustrated in Fig. 1. The calculated pairwise probability of coincidence scores for the PAC clones also indicated that 6/11 PAC clones could be ordered into two contigs using a cutoff probability of coincidence score of $<1 \times 10^{-7}$: one contig selected with DRA and DRB cDNAs (PACs D2, F2, and G7) and one contig selected with DOB (PACs 6B1, 7B2, and 2A1) (Fig. 1). In addition, the fingerprint analysis allowed us to assign the DRB-positive PAC F4 to the interval between BACs 69c13 and 244e2 and PACs F20 (isolated using Ring3) and F23 (isolated using DPA) to the interval between BACs 40m13 and 131i17 (Fig. 1).

To determine the size of the BAC/PAC contig, we first estimated the size of each restriction fragment in basepairs for each clone insert using the *Hin*dIII fingerprint data and determined the size of each insert (Fig. 1). The average insert size of the feline MHC class II region PAC and BAC DNA inserts calculated in this manner was 83 and 134 kb, respectively, remarkably close to the 80 and 138 kb estimated by PFGE of random PAC and BAC clones from these libraries (see above and www.chori.org/bacpac). Thus, we estimated that the error in our size estimates was ~3%. Second, using the restriction fragment size data of each clone and the number of matching restriction fragments between overlapping clones, we reconstructed the feline MHC class II BAC/PAC contig map using the estimated size data (Fig. 1). The feline MHC class II region BAC/PAC contig spans ~660 kb, in good agreement with the target size estimate of 632.5 kb based on the number of BAC clones and the average BAC insert size as determined by PFGE.

Characterization of Feline Class II Region BAC/PAC Clones

To verify the overlaps between BAC/PAC clones determined by FPC and to determine partially the gene order and gene number across the domestic cat MHC class II region BAC/PAC contig, Southern blots of *Hin*dIII-digested BAC/PAC DNAs were examined using hybridization probes for feline DRA, DRB, DOB, Tap1, DMB, and Ring3. In addition, we considered that this approach was likely to aid in: (a) identifying

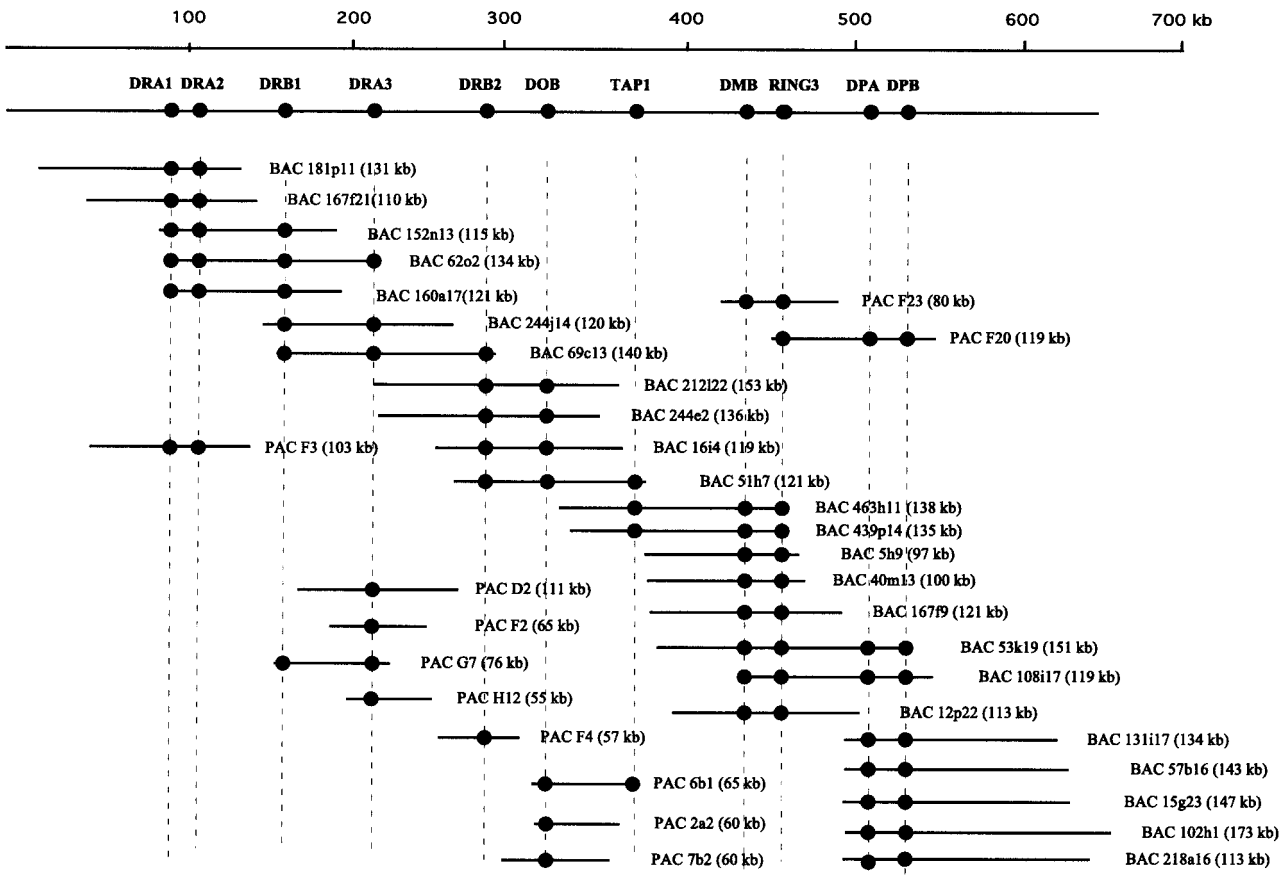

FIG. 1. BAC/PAC contig map spanning 660 kb of the domestic cat MHC class II region. Summary of BAC/PAC contig and gene mapping, incorporating fragment sizes of all clones. The top line shows the length in kilobases. The second line shows the gene locations based on marker data presented here. The lower portion shows the 24 individual BAC and 11 PAC clones and their sizes and incorporates the marker data. The classical feline MHC class II region is approximately 500 kb. Note: additional DRB gene sequences can be detected in the interval between DRA3 and DRB2 in specific BAC/PAC clones, and the number of DRB genes indicated represents a minimal estimate based on the partitioning of DRA3. Additional genes (e.g., DNA, LMP2/7, and TAP2) present in human/mouse MHC class II regions were not analyzed.

shared probe-positive restriction fragments between overlapping clones (i.e., identical haplotypes); (b) identifying probe-positive restriction fragments not shared between overlapping clones (i.e., unique haplotypes); and (c) the assignment of PAC clones to the BAC contig that FPC was unable to map based on fingerprints. The results are described in detail below according to region/locus and are summarized in Table 1 and Fig. 1.

DR region—DRA. Southern blot hybridization of the *Hin*dIII fingerprints with feline class II DRA confirmed the FPC predicted overlaps for five BAC clones (181p11, 167f21, 62o2, 160a17, and 152n13) in that each clone contained an identical (or nearly identical—BAC 152n13) set of DRA hybridizing restriction fragments (3.9, 2.9, 2.3, and 1.0 kb; Fig. 3B). PAC F3, which did not overlap significantly with any clone in the database, contained an identical set of *Hin*dIII fragments (3.9, 2.9, 2.3, and 1.0 kb; Fig. 2B), indicating that PAC F3 must map to the interval defined by BACs 181p11, 167f21, 62o2, 160a17, and 152n13. In addition six clones, two BACs (244j14 and 69c13), and four PACs (D2, F2, G7 and H12) also contained 2.3- and 1.0-kb DRA-positive restriction fragments (Figs. 2B and 3B) even though the FPC contig map indicated that BACs 244j14 and 69c13 did not significantly overlap BACs 181p11 and 167f21 (Fig. 1). Since a total of 12 clones had been identified containing the 2.3- and 1.0-kb DRA-positive restriction fragments from an ~7× genomic screen, this suggested the possibility of a duplication.

To examine the organization of DRA sequences in the domestic cat MHC in greater detail, BAC/PAC DNAs were digested with *Bam*HI, electrophoresed through 0.5% agarose, transferred to Duralon-UV, and hybridized with DRA. As shown in Figs. 4A and 4B, BACs 181p11, 167f21, 62o2, 160a17, and 152n13 contained two *Bam*HI fragments (16 and 22 kb) positive for DRA as did PAC F3. BACs 62o2, 244j14, and 69c13 contained a larger ~29-kb DRA-positive *Bam*HI fragment as did PACs D2, F2, and G7. PAC H12 had a unique, 18-kb DRA fragment. The presence of all three DRA *Bam*HI fragments (16, 22, and 29 kb) in 62o2 confirmed the presence of three sets of DRA gene sequences in the DR region of the domestic cat. Finally, partial nucleotide sequence analysis of PAC F2 (Yuhki *et al.*, manuscript in preparation) indicated that the DRA sequences represented by the 2.3- and 1.0-kb *Hin*dIII fragments constituted a complete DRA gene, and therefore, the additional DRA signals observed

TABLE 1

Comparison of BAC/PAC Clone Hybridization Patterns Using Feline MHC Class II Region Probes

	DRA 3.9	DRA 2.9	DRA 2.3	DRA 1.0	DRB 20.0	DRB 4.7	DRB 1.9	DRB 1.1	DRB 13.7	DRB 16	DRA 2.3*	DRA 1.0*	DRB 1.4	DRB 5.7	DRB 0.7	DRB 7.9	DOB 4.3	DOB 0.5	TAP1	DMB 6.2	RING3 2.2	DPA 5.0	DPB 7.1
181p11																							
167f21																							
152n13																							
62o2																							
160a17																							
244j14																							
69c13																							
212122																							
244e2																							
16i4																	4						
51h7																							
439p14																							
463h11																							
5h9																							
40m13																							
12p22																							
167f9																							
108i17																							
53k19																				7.3			
57b16																							
131i17																							
15g23																							
102h1																							
F3																							
H12																							
G7																							
F2																							
D2																							
F4																							
7B2																							
2A2																							
6B1																							
F23																							
F20																							
Total	6	5	6	6	2	2	3	6	5	2	7	7	3	5	6	6	8	8	4	9	10	7	7

Note. Summary of hybridization data of feline MHC class II region BAC/PAC DNAs with feline class II region probes. The left column shows the relative order of BAC and PAC clones based on FPC, and the top row shows sizes of restriction fragments (marker) detected using the probe indicated (i.e., DRA, DRB, and DOB (4.2 and 0.5 kb), TAP1 (9 kb), DMB (8.2 kb except for 53k19, which was 7.3 kb), Ring3 (9.5-kb *Bam*HI), DPA (5.0 kb detected using DRA cDNA), and DPB (7.1 kb detected using DRB)).

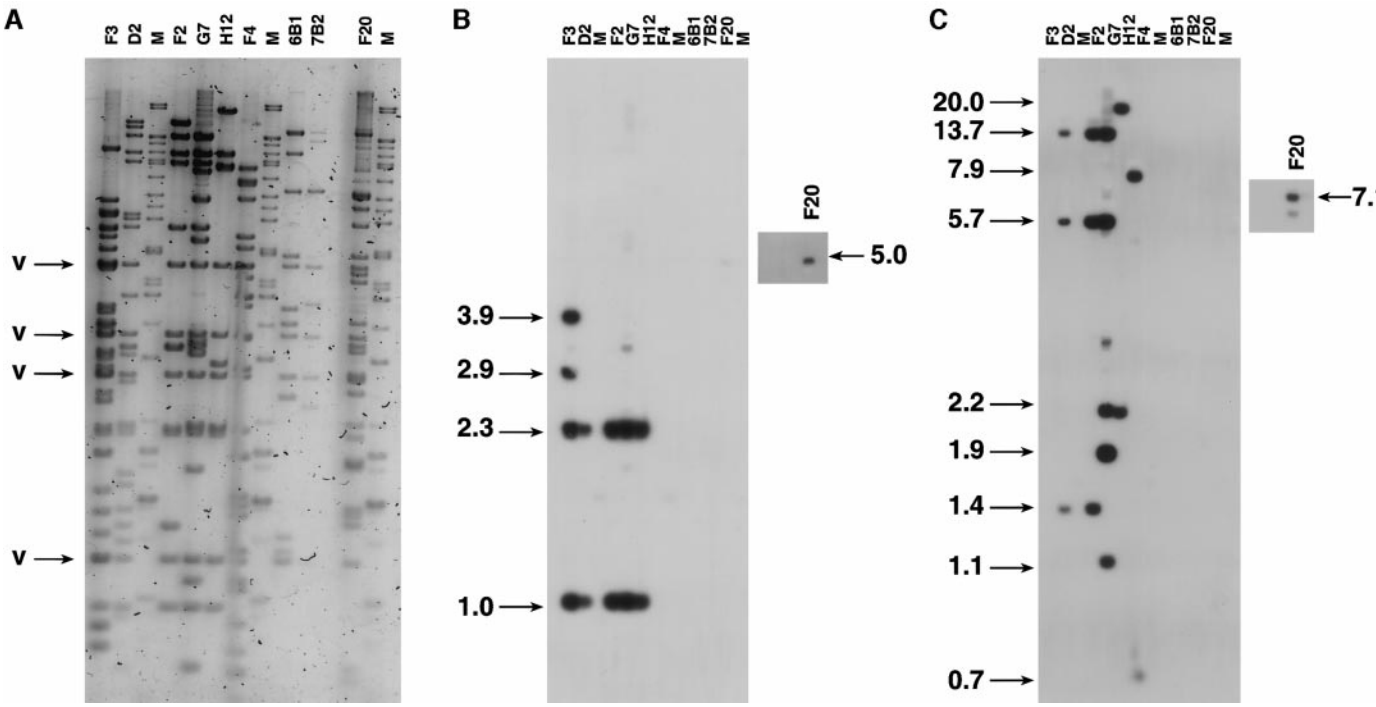

FIG. 2. Hybridization analysis of DR-positive PAC clones from the feline major histocompatibility complex class II region. (**A**) PAC DNAs (~500 ng) were digested with *Hin*dIII, fractionated through 1% agarose, stained with SYBR green, and imaged using a Hitachi Fluorimager as described under Materials and Methods. Lane **M,** markers (St. Louis Standards). The *Hin*dIII fingerprints were then transferred to nylon membranes and hybridized with ^{32}P-labeled (**B**) feline DRA cDNA or (**C**) DRB cDNA as described under Materials and Methods. The inserts in **B** and **C** show longer exposures of the indicated region.

with BACs 181p11, 167f21, 62o2, 160a17, and 152n13 and PAC F3 presumably represent two additional DRA genes. Therefore, we conclude that the domestic cat has three DRA genes: DRA1 (3.9- and 2.9-kb *Hin*dIII fragments), DRA2 (2.3- and 1.0-kb *Hin*dIII fragments), and DRA3 (2.3- and 1.0-kb *Hin*dIII fragments/29-kb *Bam*HI fragment).

DR region—DRB. Southern blot hybridization of the *Hin*dIII fingerprints with feline class II DRB cDNA identified nine BAC clones (62o2, 160a17, 152n13, 244j14, 69c13, 212122, 244e2, 16i4, and 51h7) and five PAC clones (F2, D2, G7, H12, and F4) that contained strong DRB hybridization signals (Figs. 2C and 3C). Five BACs (62o2, 160a17, 152n13, 244j14, and 69c13) and one PAC (G7) displayed a common 1.1-kb DRB-positive restriction fragment, but other than BACs 62o2 and 160a17 (that had an identical DRB hybridization pattern: 4.7, 1.9, and 1.1 kb) and PACs D2 and F2 (that had an identical DRB hybridization pattern: 13.7, 5.7, and 1.4 kb), all differed in the number and sizes of the remaining DRB-positive restriction fragments as summarized in Table 1. For example, BAC 152n13, which had a high probability of coincidence score with both BACs 62o2 ($P = 4e^{-13}$) and 160a17 ($P = 7e^{-12}$), shared a nearly identical DRA hybridization pattern (Fig. 3B), and thus overlapped with them (Fig. 1), showed unique DRB positive restriction fragments (20 and 13.7 kb) not found in either BACs 62o2 or 160a17 (Fig. 3C), presumably reflecting allelic variation in DRB. Similarly, BACs 244j14 and 69c13 had a significant probability of coincidence score ($P = 9 \times 10^{-9}$) and shared identical DRA hybridization patterns and common DRB fragments (16, 5.7 and 1.1 kb), but BAC 244j14 displayed additional DRB restriction fragments (13.7, 6.7, 6.0, and 1.4 kb) not observed in BAC 69c13, presumably reflecting allelic variation in DRB gene sequences and/or gene number. Five BAC clones (69c13, 212122, 244e2, 16i4, and 51h7) and one PAC clone (F4) contained 7.9- and 0.7-kb DRB-positive restriction fragments, confirming the overlaps identified in the FPC contig map. The complexity of the DRB hybridization data prevented us from drawing any firm conclusions as to the precise number of DRB genes in the domestic cat. However, taking into account only the most frequently observed DRB-positive *Hin*dIII restriction fragments in both libraries, (a) the 1.1-kb fragment (BACs 62o2, 160a17, 152n13, 244j14, 69c13 and PAC G7) defining DRB gene 1, (b) the 7.9-/0.7-kb fragment (69c13, 212122, 244e2, 16i4 and 51h7 and PAC F4) defining DRB gene 2, as well as (c) the 29-kb DRA-positive *Bam*HI restriction fragment (BACs 62o2, 244j14, 69c13, and PACs D2, F2, and G7) defining DRA gene 3, the simplest order is DRB1–DRA3–DRB2, indicating at least two DRB genes in the domestic cat class II region (Fig. 1). The results presented are consistent with the allelic variation observed in DRB cDNAs and PCR products from the domestic cat and suggest that the number of DRB genes in the domes-

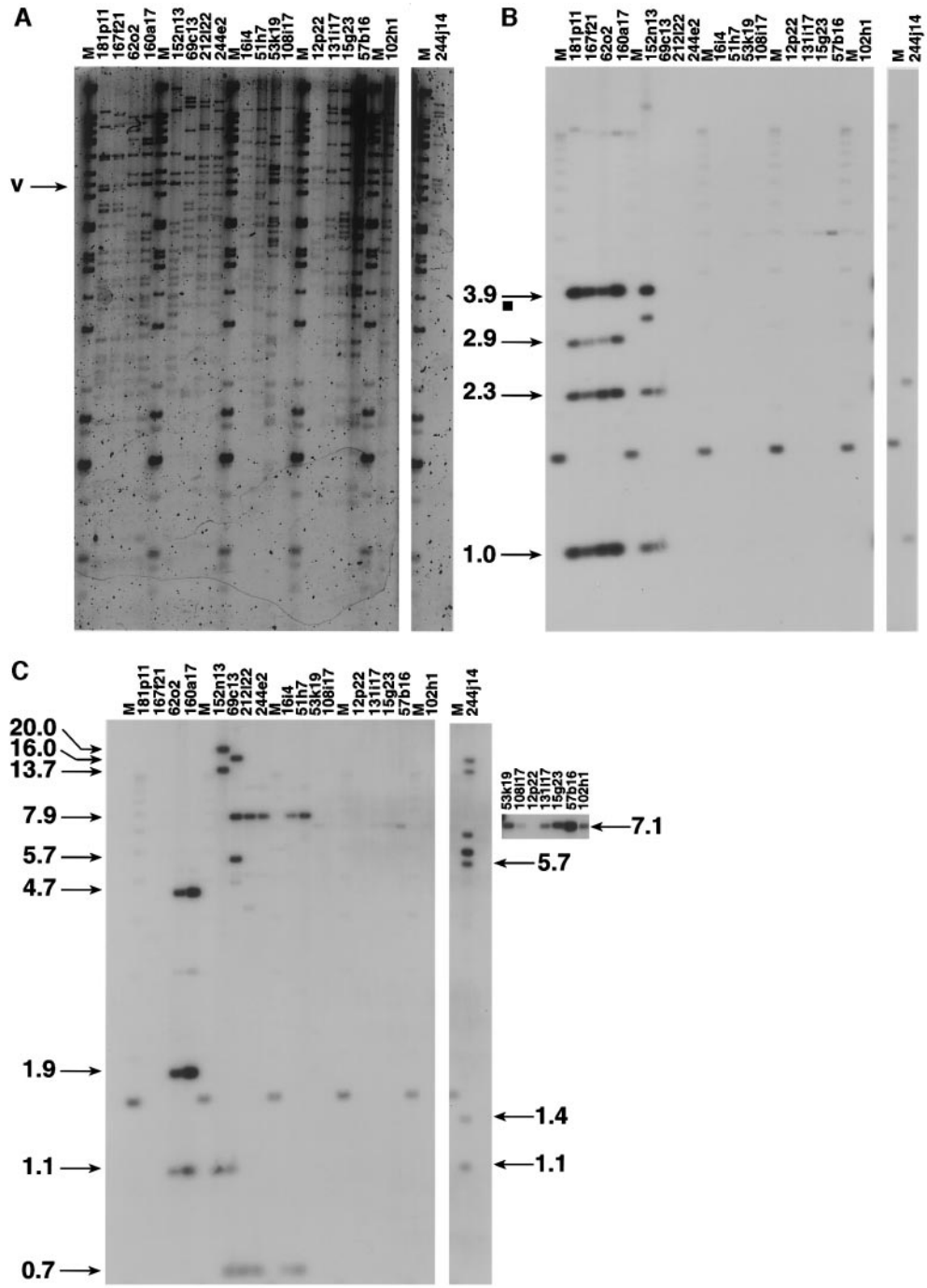

FIG. 3. Hybridization analysis of DR-positive BAC clones from the feline major histocompatibility complex class II region. (**A**) BAC DNAs (~100 ng) were digested with *Hin*dIII, fractionated, and stained as described in the legend to Fig. 2. Lane **M,** markers (St. Louis Standards). The *Hin*dIII fingerprints were then transferred to nylon membranes and hybridized with either (**B**) feline DRA cDNA or (**C**) DRB cDNA as described in the legend to Fig. 2. The insert in **C** shows a longer exposure of the indicated region.

tic cat MHC class II region varies between haplotypes as has been observed in the human DRB region (Trowsdale *et al.*, 1991).

DOB—Ring3. Using PCR products specific for feline DOB, TAP1, DMB, and Ring3 as probes in Southern blot or colony hybridizations, we sought to identify BAC/PAC clones containing each of these markers. Even though the fingerprinting data failed to map the PAC contig composed of 6B1, 2A2, and 7B2 to the BAC/PAC contig, hybridization of DOB to *Hin*dIII fingerprints demonstrated that all three PACs were positive for DOB, as were four BAC clones (212122, 244e2, 16i4, and 51h7), and all displayed hybridizing fragments of 4.3 and 0.5 kb (except BAC 16i4, which displayed a 4.0-kb fragment). In addition, PAC 6B1 and three BACs (51h7, 439p14, and 463h11) were positive for TAP1 (51h7 and 6B1 displayed 9- and 13-kb *Hin*dIII fragments, respectively). Therefore, the PAC

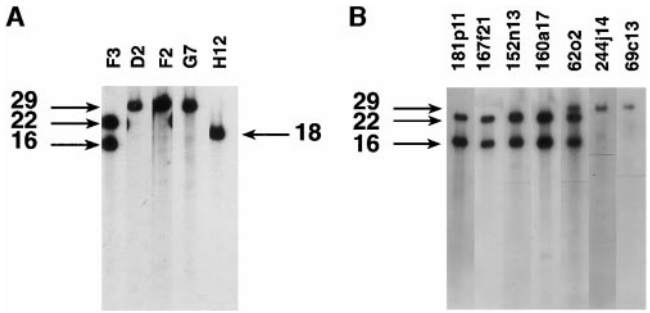

FIG. 4. Hybridization of DRA to *Bam*HI-digested DR containing BAC/PAC DNAs. BAC/PAC DNAs were digested with *Bam*HI, fractionated through 0.5% agarose (MP, Boehringer Mannheim), stained, analyzed, transferred to nylon membranes, and hybridized with ^{32}P-labeled DRA cDNA as described in the legend to Fig. 2.

contig (6B1, 2A2, and 7B2) maps to the interval spanning BACs 212122, 244e2, 16i4, 51h7, 439p14, and 463h11 and contains DOB and TAP1 (Table 1 and Fig. 1). Interestingly, BAC 51h7 was 121 kb in length and contained the three markers DRB2 (DRB7.9 and 0.7), DOB (DOB4.3 and 0.5), and TAP1 (9 kb). The completed nucleotide sequence of PAC 6B1 showed that DOB and TAP1 were ~60 kb apart (Yuhki *et al.*, manuscript in preparation). Therefore, assuming the same distance between DOB and TAP1 in BAC 51h7, DRB2 and DOB must also be ~60 kb apart, indicating that the domestic cat DQ region, if present, is considerably smaller than that of the human DQ, which spans ~200 kb, consistent with our failure to detect DQ-related transcripts in domestic cat cDNA libraries (Yuhki *et al.*, 1997).

As shown in Table 1, DMB hybridized with a total of eight BAC clones (439p14, 463h11, 5h9, 40m13, 167f9, 53k19, 108I17, 12p22) and PAC F23 (detected by hybridization to an 8.2-kb *Hin*dIII restriction fragment except for 7.3 kb in 53k19). Ring3 hybridized to a 9.5-kb *Hin*dIII fragment in five BACs (5h9, 40m13, 167f9, 108I17, 12p22) and two PACs (F23 and F20). Even though the probability of coincidence scores for F20 and F23 were above the cutoff, both clones were positive for Ring3 and, therefore, must be minimally overlapping.

DP region—DPA and DPB. Since the class II genes of the mammalian MHC are thought to have evolved from a common ancestor, it was not surprising that both DRA and DRB cDNAs hybridized weakly to BAC/PAC clones lying outside the DR region. As shown in Figs. 2B and 2C, *Hin*dIII-digested PAC F20 displayed a 5-kb fragment that hybridized with DRA and a 7.1-kb fragment that hybridized with DRB. Partial nucleotide sequence analysis of PAC F20 indicated that feline DPA and DPB genes were located within 5.0- and 7.1-kb *Hin*dIII fragments, respectively. Identically sized DRA and DRB hybridizing restriction fragments were also observed with BACs 108I17, 53k19, 57b16, 131I17, 15g23, and 102h1 digested with *Hin*dIII (Figs. 3B and 3C). Since PAC F20 also displayed a high probability of coincidence scores with BAC clones in this interval, we interpret these DRA and DRB hybridization signals as cross-hybridization to DPA and DPB genes, respectively. As shown in Fig. 1, the gene mapping experiments taken as a whole confirmed the overlaps between BAC and PAC clones determined by FPC, increased the number of PACs mapped to the contig, and established a gene order of DR–DOB–TAP1–DMB–Ring3–DP across the domestic cat MHC class II region BAC/PAC contig.

Fluorescence in Situ Hybridization of Feline Class II PAC Clones

Figure 5 shows a fluorescence *in situ* hybridization of PACs F2 (DRA–DRB), H12 (DRA–DRB), and F20 (DPB–Ring3 to metaphase spreads of domestic cat chromosomes. All three clones showed localization to the pericentromeric region of cat chromosome B2, establishing visually that the domestic cat DR and DP regions are contiguous on chromosome B2. These results are consistent with previous mapping of the feline class II region in somatic cell hybrids and confirm more recent mapping of DRA, DOB, and DPA genes in the domestic cat radiation hybrid panel (Yuhki and O'Brien, 1988; Murphy *et al.*, 2000).

DISCUSSION

In this article, we describe the construction of a 0.66-Mb BAC/PAC contig map spanning the class II region of the domestic cat MHC consisting of 24 BAC and 11 PAC clones that map to chromosome B2 based on FISH analysis of DR- and DP-containing PAC clones. The BAC and PAC clones were first ordered into a minimal tiling path using *Hin*dIII fingerprints and analyzed using FPC software (Soderlund *et al.*, 1997) as described by Marra *et al.* (1997, 1999). Second, the *Hin*dIII fingerprints were then used as tools in hybridization analyses with genetic markers (in conjunction with partial nucleotide sequencing) to verify the overlaps determined by FPC and to order 10 genes within the BAC/PAC contig. This approach was also useful because it allowed us to obtain new information concerning: (a) the number and order of DRA and DRB genes in the domestic cat that have evolved by gene duplication and were unable to be resolved using the domestic cat radiation hybrid panel; (b) possible allelic and haplotypic variants; and (c) the size of the feline MHC class II region. The size of the BAC/PAC contig, based on the order of clones established by FPC and the insert sizes based on *Hin*dIII fingerprints (660 kb), agrees well with the estimate based on the number of clones from a 4.8× BAC genomic screen and the average insert size of randomly selected BACs determined by PFGE (632.5 kb).

The results presented here extend recently established gene order across the feline MHC (MOG–Class I–TNFA–CSNK2B–C4A–DRA–DOB–DPA–RPS18) determined using the domestic cat radiation hybrid panel

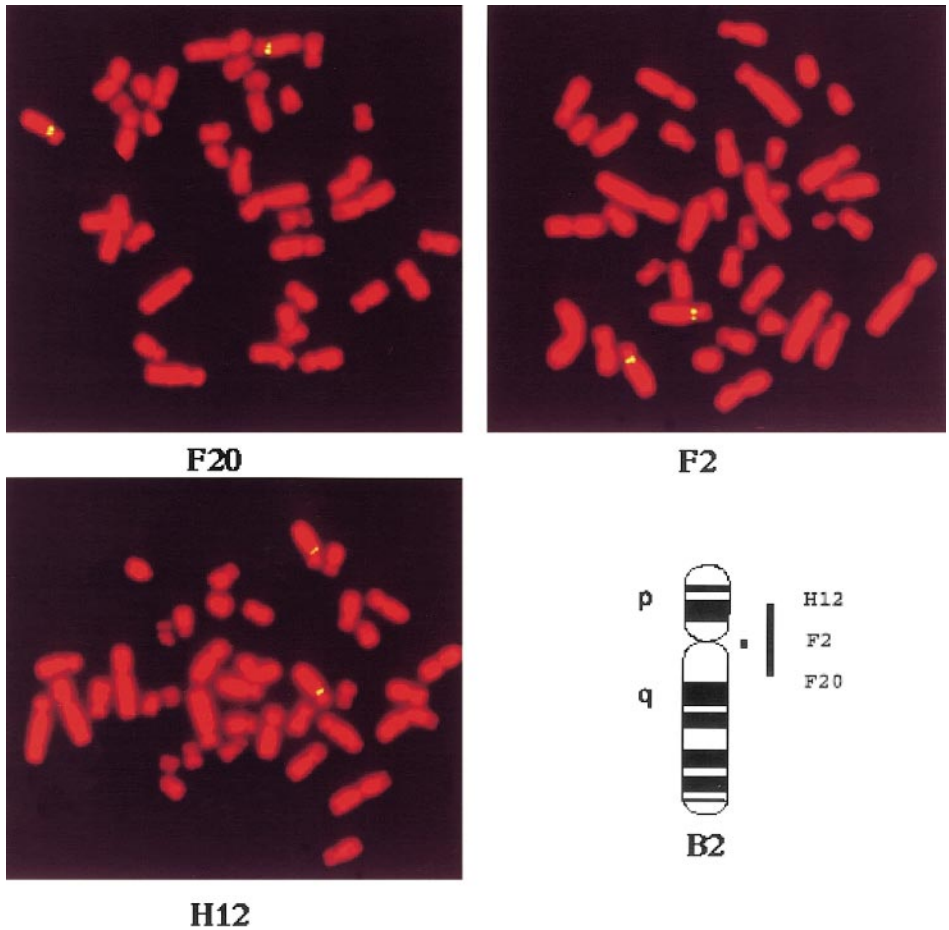

FIG. 5. Localization of domestic cat MHC class II PAC clones to chromosome B2. PAC DNAs were labeled with biotin, hybridized to metaphase spreads of domestic cat chromosomes, and visualized as described under Materials and Methods. All three PAC clones localize to the pericentromeric region of domestic cat chromosome B2.

(Murphy *et al.*, 2000) by the addition of seven new markers in the class II region. Five genes (DOB, Tap1, DMB, Ring3, DPA, and DPB) were mapped to a single locus in the domestic cat with an order identical to that in the human MHC (although the precise orientation of DPA and DPB remains to be determined). The DP region in human and anthropoid primates consists of a pair of pseudogenes (DPB2 and DPA2) and a pair of functional genes (DPB1 and DPA1) (Bontrop *et al.*, 1999; Grahovac *et al.*, 1993). However, using DR cDNA probes in Southern blot hybridizations, complex patterns of DRA and DRB hybridization signals that are localized within an ~200-kb region were revealed. Three DRA genes were identified (DRA1, 2, and 3) with nearly identical hybridization patterns between the two libraries, and at least two DRB genes flanking DRA3 with distinct hybridization patterns between libraries were identified. The patterns of DRB hybridization to BAC and PAC clones were complicated, presumably due to restriction fragment length polymorphisms, insertions, and/or deletions of DRB sequences reflecting allelic variation (e.g., compare BAC 62o2 and BAC 152n13) and perhaps variation in gene number (e.g., compare BAC 69c13 and BAC 244j14). Of the four possible DR region haplotypes represented within the two libraries, the results are consistent with one (e.g., BAC 62o2 and BAC 69c13/244j14) or perhaps two (e.g., BACs 62o2/152n13 and BACs 69c13/244j14) DR haplotypes being complete. These results are indicative of genetic variation between DRB genes in the domestic cat and most mammalian species examined to date; however, the precise number of DRB genes and haplotype identification will require nucleotide sequencing of the representative clones. Nevertheless, these results are consistent with and extend those of Yuhki and O'Brien (1997), who estimated minimally two DRA and three DRB genes in the domestic cat per haploid genome based on cDNA and PCR sequencing of gene fragments from single individuals.

The close proximity of DRB to DOB within ~60 kb of each other in the domestic cat suggests that the region containing DQA and DQB genes in the human, which spans an interval of ~200 kb, is not represented in the domestic cat class II region, which accounts for the smaller size of the feline DRA–DPB interval (~500 kb) versus the human region (~700 kb). In fact, preliminary nucleotide sequencing of BAC 244e2 indicates that DQ genes are not present in the DRB–DOB interval in the domestic cat class II region (Yuhki, manu-

script in preparation), consistent with our previous inability to detect evidence of DQA and DQB from domestic cat cDNA libraries (Yuhki *et al.*, 1997). In contrast, all primates and mammalian species examined to date express both DQA and DQB genes, making the apparent deletion of DQ unique to the domestic cat. Other than DQ segment deletion and DRA gene expansion, the gene order across the MHC class II region is identical to that in the human; DPB/DPA–Ring3–DMB–TAP1–DOB–DRB/DRA. However, other genes present in human/mouse MHC class II regions (e.g. DNA, DMB, TAP2) were not analyzed and a more definitive description of the gene content and gene order of the feline MHC class II region must await direct nucleotide sequencing of the BAC/PAC contig.

The feline genomic PAC library described here has an average insert size of 80 kb and a 2.5× genome coverage. The feline BAC library constructed by de Jong and colleagues (Wang *et al.*, manuscript in preparation) using improved techniques for large-insert cloning is better suited for large-scale mapping projects because of its larger insert size, greater coverage, and the increased sensitivity of screening arrayed libraries. Nevertheless, the feline PAC library will be useful for analysis of genetic variation between individuals as described here for the feline class II region and other genomic segments where diversity is high.

Finally, the BAC/PAC contig described here provides a minimal tiling path of clones for large-scale nucleotide sequence analysis of the domestic cat MHC class II region and comparative analysis of the mammalian MHC class II region using nucleotide sequences from human and mouse large-scale MHC sequencing projects. The development of detailed comparative large-scale nucleotide sequences from model, agricultural, and domestic mammals, particularly in dynamic regions of the human genome such as the MHC, may provide clues for the discovery of evolved adaptations to infectious/hereditary disease and mammalian genomic principles.

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